BIOLOGICAL BULLETIN

THE INFLUENCE OF THE NUCLEUS ON THE BEHAVIOR OF AMŒBA.¹

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INTRODUCTION.

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In the course of experimental work on *Amæba* in November, 1914, marked differences were observed in the behavior of the different parts of specimens cut in two. At the suggestion of Dr. S. O. Mast, an attempt has been made to ascertain whether these differences depend upon the nucleus and, if so, in what respects. I wish here to acknowledge my great indebtedness to Dr. Mast for his helpful and constructive suggestions and for his kindness in supervising both the experimental work and the writing of this paper.

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Gruber (1912) found that parts of *Amæba proteus* containing a nucleus behave very much like normal specimens, but that parts without a nucleus behave very differently; yet, in spite of this, he held that the nucleus in general has no influence upon protoplasmic movement. Hofer (1890) in observations on the same species, obtained results similar to those of Gruber. He asserts (p. 118) that movements in the parts with the nucleus are similar to those in normal specimens, as are also those in the other parts for a period of 15–30 minutes after division, but that later the movements in the parts without a nucleus differ from those in normal specimens in rate of locomotion, in regularity of movement, and in the number and length of pseudopods.

Hofer holds that these differences might be considered to be due either to the injury received during the operation or to the influence of the nucleus. But he maintains that, since any injury sustained from the operation affects both fragments alike, the operation could not be considered the cause of the observed difference in behavior. He consequently concludes that the real cause is to be found in the nucleus. He holds, however, that the influence of the nucleus may be conceived to be direct or indirect; that is, that behavior may be due to an impairing of the elementary functions (such as digestion, respiration, and excretion) controlled by the nucleus. But Hofer found that the process of digestion in the absence of the nucleus continues for several days after division; that respiration takes place in the absence of the nucleus; and that excretory functions in enucleated segments continue till death. He therefore comes to the conclusion that the nucleus secretes a chemical substance and that behavior is controlled through this. He thinks a certain amount of this substance is stored up in the different parts of the protoplasm, and that the normal movement of the enucleated segments for 15-30 minutes after the operation is due to the influence of the substance thus stored. Hofer maintains that, since movement occurs in parts without a nucleus, cytoplasm has the power of movement; but since the movement in these parts is more irregular and haphazard than it is in nucleated parts, he holds that the nucleus must have a regulatory function. In other words, he thinks the nucleus serves as a regulatory "centrum" for behavior.

Verworn (1909) agrees with Hofer in holding that there is a difference in the behavior of parts of Amaba with and parts without a nucleus, but does not agree with him in the conclusion that the nucleus exerts a direct influence on the movement; that is, he does not think that the nucleus is a regulatory "centrum" for movement.

Judging from the results of my experiments, it is clear that there are distinct differences in the behavior of parts of Amwbawhich contain and parts which do not contain a nucleus. Differences in such parts have been observed in the character of movement, in the accuracy of orientation in light and in the rate of locomotion. It is clear, also, that these differences are traceable to the nucleus.



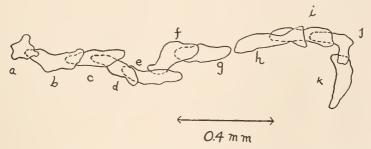


FIG. I. Camera sketches showing changes in form and the characteristic movements in the nucleated and enucleated parts of an amœba immediately after being cut in two. The arrows indicate the direction of movement. I-3, enucleated part; a-k, nucleated part; mm, projected scale. The sketches of the enucleated part were made at five-minute intervals; those of the nucleated part at one minute intervals. Note that the nucleated part progressed regularly in a given direction, and that the enucleated part changed its form and position only slightly.

MATERIAL AND METHODS.

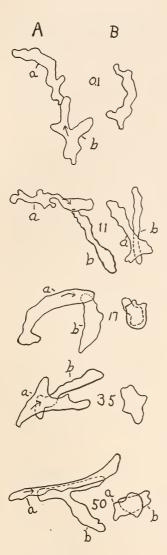
The specimens used for the most part in this work appeared in a battery jar containing an old paramecium culture. They corresponded to descriptions of *Amæba proteus* and were rather sensitive to light. Nucleated and enucleated parts were obtained by cutting specimens in two with finely drawn glass rods. The cutting was done under a binocular. The parts of each individual were usually enclosed under a cover-glass by means of a ridge of vaseline so applied along the edge as to support the cover-glass and prevent drying. Thus the parts had entire freedom of movement and both were continuously subjected to the same environment; and, under these conditions, they were studied, observations being made both with a compound microscope and a binocular. Under the sealed cover-glasses the divided amœbæ did remarkably well, the nucleated parts living on an average of approximately ten days, *i. e.*, practically as long as normal specimens under the same conditions, and the enucleated parts about half as long. In the following descriptions, the two parts will frequently be designated fragments or segments.

MOVEMENT.

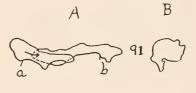
Normal Specimens.—In the process of locomotion in normal individuals of the species studied, pseudopods usually appear alternately on the two sides of the organism near the anterior end. A pseudopod appears, for instance, on the right, elongates and enlarges by the flow of protoplasm into it until it constitutes the main portion of the animal; then from this there is formed a new pseudopod on the left side. This, in turn, elongates and enlarges, after which a new pseudopod again forms on the right side, etc. Thus the organism takes a zigzag course.

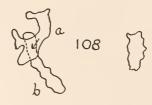
Fragments.—In general it was found that the movement in fragments containing a nucleus is substantially like the movement exhibited in normal specimens, and, in some instances, for a period of 10–20 minutes after division, it was found to be similar in enucleated parts. Usually, however, the movement is strikingly different in such parts, it being slow and irregular, and frequently accompanied by contractions. The pseudopods, all

FIG. 2. Series of camera sketches illustrating the difference in the movement of nucleated and enucleated segments of $Am\alpha ba$. The former is shown in columns A, the latter in columns B. The numerals between the columns indicate approximately the intervals of time, in hours, between the cutting of the am α ba and the production of the adjoining sketches in the two columns. The two sketches, *a* and *b*, in every case were made about one minute apart. They show the changes in position and in form of the segments during this time. Whenever there is but one sketch opposite the numerals, it indicates that there was no change in the organism. *a*, first position; *b*, second position, arrows, direction of movement, *mm*, projected scale.

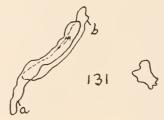


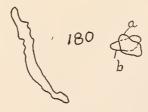












 \bigcirc 228

0.4 mm

⇒

of which are usually small, are not formed alternately as they are in the parts containing the nucleus. Neither do they always occur near the anterior end. These differences in movement in the two segments are illustrated in Figs. 1 and 2.

The nucleated part represented in Fig. I formed pseudopods within one minute after division. At three minutes it was attached to the substratum and exhibited the coördinated movement characteristic of a normal individual (Fig. 1, a, b, c, etc.). This fragment was observed every three to five minutes for an hour, and the movement was found to be essentially the same throughout the entire period. The enucleated part of this individual on the contrary, became globular immediately and remained so for approximately five minutes during which time a number of small short pseudopods were directed outwards in all directions from the body. These became fewer and larger, and the entire body elongated in the direction of one of the larger pseudopods. An attachment of the body to the substratum was then formed and regular movement followed for about one minute. There was no subsequent locomotion or "streaming movement"; the organism, however, changed its shape by frequent contractions of the cytoplasm. These changes continued for an hour when the experiment was brought to a close (Fig. 1, 1-3). Throughout the entire period, the movement in the enucleated part was very much slower than that in the nucleated part.

Essentially all of these characteristic differences in the movements of the two fragments in question are further elucidated in the sketches reproduced in Fig. 2. By referring to this figure it will be seen that there was considerable locomotion in the nucleated part and that pseudopods were formed more or less regularly on the opposite sides of the segment; while in the enucleated segment there was extremely little locomotion and pseudopods were formed irregularly.

Figs. 1 and 2 are typical illustrations of the behavior observed in all of the specimens studied—forty in number. The movement in all nucleated parts of these specimens was like that in normal specimens and the movement in all enucleated parts was quite different from that in normal specimens. The behavior of 17 pairs of these parts taken at random is briefly summarized in Table I. By referring to this table, in which observations on locomotion are recorded for 13 of the 17 individuals, it will be seen that locomotion occurred in 13 of the nucleated parts, while there was no locomotion in the enucleated parts. Other matters in this table will be considered later.

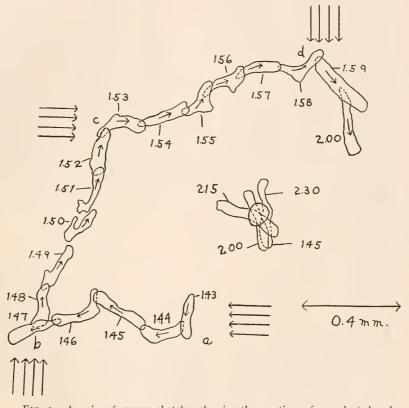


FIG. 3. A series of camera sketches showing the reactions of a nucleated and an enucleated part of an amœba in a horizontal beam of light. a-d, nucleated part; 1.45-2.30 enucleated part; large arrows, beam of light; small arrows, direction of movement; 1.43-2.00, time at which sketches of nucleated part were made; 1.45-2.30, time at which sketches of enucleated part were made; mm, projected scale. Note that the nucleated segment oriented fairly definitely; at a (1.43) the rays of light were at right angles to the moving segment; at 1.44 the segment had turned and become directed from the source of light. A similar response occurred at b, c, and d after the direction of the rays of light had been changed in each case. Note also that the enucleated part did not orient in the light. Both parts were continuously in the same field and were subjected to the same changes in illumination.

sus; the position the ments were studied, emained attached to me attached and did	Behavior.	
Table showing the relation between the reactions of fragments of $Amwbu$ and the presence or absence of the nucleus; the position the fragments occupied in the intact specimens; the size of the fragments; the length of time the various pairs of fragments were studied, and the attachment of the parts to the substratum. Note that the nucleated parts, regardless of size or position, remained attached to the substratum and responded essentially like normal specimens, and that the enucleated parts rarely, if ever, became attached and did not respond like normal specimens.	Relation of Parts to Substratum.	
ments of Ama e fragments; t i that the nucle cimens, and th	Length of Time Studied (in Hours).	
actions of frag the size of th tratum. Note ce normal spee	Relative Size of Parts.	
in between the re- intact specimens; parts to the subsi- led essentially fil- imens.	Designation of the Individuals Contents of Position Occupied Relative Size Length of Time by the Two Parts. Of Parts. In Hours, In Hours	
ing the relation apied in the in nuent of the J a and respond ce normal spec	Contents of Parts.	
Table showing the relation betw fragments occupied in the intact s and the attachment of the parts to the substratum and responded ess not respond like normal specimens.	Designation of the Individuals Cut into Two Parts.	

TABLE I.

t respond li	t respond like normal specimens.	climens.				
esignation of le Individuals ut into Two Parts.	Contents of Parts.	Position Occupied by the Two Parts in Intact Amœba.	Relative Size of Parts.	Length of Time Studied (in Hours).	Relation of Parts to Substratum.	Behavior.
	Nucleus.	Anterior end.	Slightly larger.	34	Part attached I min. after division.	Locomotion.
I	No nucleus.	Posteriór end.	0		Attached one min. after division. At- tachment lost after 5 mins. Regained and lost in first 20 min. after division.	Slight motion.
0	Nucleus.	Anterior end.	Larger.	1/2	Attached after 2 min. Did not aftach.	Locomotion. Became snherical.
3	Nucleus.	Anterior end	Equal.	1/6	Attached soccar after division. Attached slightly.	Locomotion. No locomotion.
4	Nucleus.	Posterior end.	Equal.	1/5	Attached 2 min. after division. Did not attach.	
ĩ	Nucleus.	Anterior end.	Equal.	14	Attached 2 min. after division and re- mained so.	
9	No nucleus. Nucleus. No nucleus.	Posterior end. Posterior end. Anterior end.	Equal. Equal.	1 ½3	Did not attach. Attached. Attached 30 min. after division. At-	Movement normal.
r	Nucleus	Anterior end.	Larger	I	tachment broken by gentle blowing on the water. Did not attach again. Attached 7 min. after division and re-	Irregular motion.
- 0	No nucleus.	Posterior end.	16 larger		mained so. Did not attach. Attached 2 min. after division and re-	Movement normal. Became spherical.
0	No nucleus.	Posterior end.	2 m P m	4	mained so. Attached slightly 25 min. after division;	Movement normal.
					lost at 30 min. No further attachment.	Pseudopods irregu- larly formed.

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Behavior.	Locomotion.	:	Irregular motion.				Locomotion.			Locomotion.	Irregular motion.
Relation of Parts to Substratum.	Attached 2 min. after cutting. Attach- ment broken 5 min. later. Attached again in 1 min. and remained so.	Attached 2 min. after division. Un- attached 5 min. later. Attached at 20 min. after division. Unattached at 25 min. and	remained so. Did not attach for an hour.	Did not attach for an hour. Remained attached after first 10 min.	Unattached for I hour when it slightly attached. Remained attached I Smin. No	further attachment. Attached 5 min. after cutting. Attach-	ment broken 3 min. later. Attached again ro min. after division, and remained so.	Attached 15 min. after division. Attachment broken 3 min. later. It did not attach	again. Attached 5 min. after division. Attach-	after 2 min. and remained so. Attached 5 min. after division. Attach-	ment broken 3 min. later; then formed again after 20 min. This was lost 2 min. later. No further attachment.
Length of Time Studied (in Hours).	I		I	1 1/2		2 1/2	1		3		
Relative Size of Parts.	Equal.		Smaller.	Equal.	4	Larger.)		Equal.		
Position Occupied by the Two Parts in Intact Ameba.	Posterior end.	Anterior end.	Anterior end.	Posterior end. Posterior end.	Anterior end.	Posterior end.		Anterior end.	Anterior end.	Posterior end.	
Contents of Parts.	Nucleus.	No nucleus.	Nucleus.	No nucleus. Nucleus.	No nucleus.	Nucleus.		No nucleus.	Nucleus.	No nucleus.	
Designation of the Individuals Cut Juto Two Parts.	6		10	II	4	12			13		

TABLE I.—Continued.

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Behavior.	Locomotion.	Much contraction.	Locomotion.	Contractions. Locomotion.	No locomotion.	Contractions.
Relation of Parts to Substratum.	Attached 4 min. after cutting and re- mained so. Attached r min. after division. Un- attached at c min. after. 15 min. later	attached again for several min. No sub- sequent attachment. Attached 5 min. after division. Attach-	ment was noticed at many observations during the 52 hours. Observed at intervals of 5 min. each for a few hours, then observed every few hours.	No attachment. Attached ro min. after division and re- mained so	No attachment. Remained attached after 1 min. following	attached 5 min. after division. Remained attached for $\frac{1}{2}$ hour. No further attachment.
Length of Time Studied (in Hours).	3	52		72	72	
Relative Size of Parts.	1/3 larger.	Smaller.		Equal.	Equal.	
Position Occupied by the Two Parts in Intact Amœba.	Anterior end. Posterior end.	Posterior end.	Anterior end.	Posterior end.	Anterior end. Posterior end.	Anterior end.
Contents of Parts,	Nucleus. No nucleus.	Nucleus.	No nucleus.	Nucleus.	No nucleus. Nucleus.	No nucleus.
Designation of the Individuals Cut into Two Parts.	14	IS		16	17	

TABLE I.—Continued.

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It has been thus shown that there is a difference in the general movements of the segments containing and those not containing a nucleus. Attention is now directed to the reactions to light in such segments, especially the reactions resulting in orientation.

ORIENTATION IN LIGHT.

Normal Specimens.—It is well known that certain species of Amæba when placed in a horizontal beam of light, usually turn until they are directed from the source of light and then continue in a fairly direct course; that is, they orient and are negative. If now the position of the source of stimulation is changed so as to illuminate the organisms from the side, they again turn until they are directed from the light, that is, they reorient. In the form under consideration the reactions to light are marked and orientation is fairly precise.

Fragments.—Fragments containing a nucleus present precisely the same kind of reaction as do normal specimens when subjected to similar light conditions. These parts react readily to light; the movement is rapid; and the process of orientation is very much like that found in intact specimens. On the other hand, enucleated parts usually give no evidence whatever of orientation. Experiments were made on 25 pairs of segments. In 21 of these the nucleated parts oriented approximately as precisely as normal individuals, but in the enucleated parts no indication of orientation whatever was observed except in three instances, and in these there was only a mere suggestion of orientation. This will be described in more detail later.

Typical of the reactions in the 25 experiments mentioned above are the responses to light of the parts shown in Fig. 3. The reactions here represented, however, are somewhat more exact and definite than those observed in some of the other segments. In this case the enucleated fragment was the larger of the two and contained the contractile vacuole. Both segments were kept under the same cover-glass and were subjected to the same light conditions. A 165-C. P. tungsten lamp was placed 13 cm. distant from the reacting segment, and a piece of colorless glass absorbed the heat waves from the lamp. A compound microscope and a camera lucida were used. The temperature at the microscope was 29.5° C. The results obtained are in part recorded in Fig. 3. It may be observed from "this figure that the nucleated part moved from the light and that with each change in the direction of the rays of light, there was a corresponding change in the direction of the movement of the segment, while on the other hand, there was no indication of orientation in the enucleated fragment. The two parts were subjected to precisely the same conditions throughout the entire experiment.

As previously stated, there was usually no indication whatever of orientation in enucleated segments, and it is questionable whether any of these parts actually oriented, yet, in a number of cases, slight movement from the light occurred, and in three cases there was a change in the direction of movement with a change in the direction of the light. This movement from the light may have been a reaction to the light in the form of orientation or it may have been merely an accidental movement made without regard to the light. The facts regarding the three cases are these. One of the segments, while moving at right angles to the light, formed a pseudopod on the shaded side, and movement occurred in the direction of the pseudopod. With a change in the direction of the light, a short pseudopod was formed again on the shaded side, but at this point the fragment assumed a globular shape. In another segment movement occurred for several seconds at right angles to a horizontal beam of light, then the direction was changed, and the fragment moved from the source of stimulation. Upon a change in the direction of the light, this fragment changed the direction of its course and again moved from the light for approximately one minute and then became globular. In the third enucleated segment substantially the same reaction was observed. If these fragments actually oriented in response to the light, the process of orientation in them was essentially different from that in nucleated fragments.

There is, therefore, a difference in nucleated and enucleated parts of Amwba in their response to light as well as in the character of their movements; but there is also a difference in the rate of locomotion as will be demonstrated presently.

RATE OF LOCOMOTION.

Normal Specimens.—The rate of locomotion varies greatly in different individuals. Some specimens move nearly twice as fast as others. Quite a number of individuals were observed to move rather consistently at 0.27–0.3 mm. per minute; others moved at an average rate of 0.12–0.15 mm. per minute. There is a fairly definite relation between the size of the moving organism and the rate of movement. An animal travels a distance that is approximately two thirds the length of its body in one minute. The actual distance traveled by a large animal is greater for a given length of time than the actual distance traveled by a small animal for the same length of time under the same conditions. This is clearly demonstrated by the results recorded in Table II. By referring to this table it will be seen that the ratio of the distance traveled per minute to the length of the body of the specimen is approximately the same in all cases.

TABLE II.

Relation between size of Amwba and rate of locomotion. The average rate of locomotion was computed from five readings extending one minute each. The average length was computed from five measurements of the moving organism at intervals of one minute each. The rate was measured by projecting the moving organism on a scale with a camera lucida.

Maximum Length Obser		Average Dista per M	ance Traveled inute.	Ratio of Distance Traveled per Minute to Length of Body.	
0.36 n	ım.	0.24	mm.	0.66	
0.34	" "	0.20	6.6	0.59	
0.35	£ 5	0.23	44	0.65	
0.37	6.6	0.24	4 6	0.65	
0.21	" "	0.15	¢ 4	0.71	
0.26	" "	0.17	4.4	0.64	
0.20	6.6	0.14	6.6	0.72	
0.26	4.6	0.17	6 G	0.64	

Fragments.—In the work on the rate of locomotion in fragments, the two parts were on an average approximately the same size. Observations were made on each pair of segments at about the same time. Both were kept under the same cover-glass and in the same environment. It was found that the rate of movement in nucleated fragments, like that in normal specimens, bears a definite relation to the size of the body. Large nucleated segments usually travel greater distances in a given length of time than do small ones. This is shown in the results recorded in Table III. In this table are given the lengths of four intact specimens together with the rate of locomotion per minute, and the length of body, and the rate of locomotion in the nucleated and enucleated parts cut from the four intact specimens.

TABLE III.

Relation between rate of locomotion and length of body in four intact specimens of Amxba and in the nucleated and enucleated parts of these four specimens.

Norma	Specimen.	Nuclea	ted Part.	Enucleated Part,		
Average	Rate of Loco-	Average	Rate of Loco-	Average	Rate of Loco-	
Length of	motion per	Length of	motion per	Length of	motion per	
Body.	Minute.	Body.	Minute.	Boiy.	Minute,	
.376 mm. .355 ''	.244 mm. .230 ''	.275 mm. .210 ''	.150 mm. .150 ''	globular	negligible	
.340 "	.200 ''	.220 ''	.140 ''	.230 mm.	?	
.360 "	.240 ''	.200 ''	.128 ''	.230 ''	.07.4 mm.	

The table shows that the larger intact specimens and the larger nucleated parts travel greater distances per minute than do the smaller ones. It also shows that locomotion in the enucleated parts is very much slower than it is in the nucleated parts. This is, however, more clearly shown in Table IV in

TABLE IV.

A comparison of the rate of locomotion in millimeters per minute in the nucleated and the enucleated parts of ten different individuals.

Nucleated Parts.	Enucleated Parts.
0.15	0.002
0.13	0.074
0.13	0.010
0.18	0.020
0.13	0.008
0.15	0.110
0.16	0.000
0.19	0.006
0.17	0.006
0.13	0.004

which the rate of locomotion in nucleated and enucleated segments of the same individual is compared. Each number in the two columns of this table represents the average rate of locomotion for five trials, each continuing for one minute. The figures in the left column show the rate of locomotion in the nucleated parts; those figures immediately opposite, in the other column, show the rate of locomotion in enucleated parts. For instance, the nucleated segment of the individual first cut moved in five trials at an average rate of 0.15 mm. per minute, and the enucleated part cut from the same individual moved at an average rate of 0.002 mm. per minute. From the table one thing is strikingly evident: movement in nucleated parts is always much more rapid than that in enucleated parts; in no case does the rate of locomotion in parts without a nucleus equal that in parts with a nucleus.

Possible Causes of Differences in Behavior.

From the experiments cited above, it is evident that there are differences in the behavior of parts of Amwba with a nucleus and parts without a nucleus; differences in the character of movement, in orientation, and in the rate of locomotion. Now, to what may these differences be attributed? The two parts differed in size, in their position in the amœba cut to produce them, in the possession of a contractile vacuole and a nucleus. The difference observed in their behavior must be ascribed to some of these differences in structure.

Size.—In this work nearly 125 amœbæ were cut, each into two parts, one of which contained a nucleus. In approximately one half of these cases, the part without a nucleus was as large as or larger than the part with a nucleus. Records of the two parts of 13 of these amœbæ, taken at random, may be found in Table I. Both parts in all cases were kept under the same cover-glass and were subjected to the same or similar conditions. All of the nucleated parts behaved like normal specimens, but none of the enucleated parts, regardless of the relative size of the two parts. It appears, therefore, that differences in the size of parts do not determine the differences in behavior.

Contractile Vacuole.—The contractile vacuole was present in the nucleated parts of the 125 specimens experimented upon about as often as it was in the enucleated parts. There was no observable difference in behavior associated with the presence or absence of the vacuole. Enucleated parts with the vacuole apeared to behave in every way similarly to enucleated parts without a vacuole. The same is true for nucleated parts. The vacuole, therefore, cannot be reckoned as a determining factor in the behavior of the fragments.

Position Occupied by Fragments in the Intact Amæba.—When an amæba is cut into two parts, the part containing the nucleus may be from what was the anterior end or the posterior end of the original specimen. The behavior was carefully studied in 25 parts, 13 from the anterior and 12 from the posterior end of the intact individual. The results obtained are recorded in Table I. No evidence was obtained indicating that the reaction of the parts depends upon their location in the specimens from which they were taken. The nucleated parts from the anterior end responded precisely like those from the posterior end. Obviously, then, the position in the intact specimen is not a determining factor in the behavior of fragments.

The Nucleus.—We have demonstrated that parts of Amaba which contain a nucleus behave essentially like normal specimens, while those which do not contain a nucleus behave quite differently, and that this difference in the behavior of the parts is dependent upon neither their relative size, the presence of the contractile vacuole, nor the location of the parts in the animals which were cut to produce them. It must, therefore, in some way, be related to the nucleus. This will be considered in the following paragraphs.

THE INFLUENCE OF THE NUCLEUS UPON ATTACHMENT.

Dellenger (1906) made it clear that attachment always accompanies and is essential to efficient locomotion in *Amæba* and *Difflugia*. Twenty-five of my experiments on fragments of *Amæba*, in which observations were made on attachment, demonstrate quite clearly that nucleated fragments are usually continuously attached to the substratum, and that enucleated fragments are rarely attached. In these experiments some specimens were observed for only ten minutes; others for a very much longer period of time, the maximum being 72 hours. Records of attachment or unattachment in all were made at definite intervals. The following detailed description of the results obtained in the study of one pair of segments taken from the same specimen is typical of all. In this case the parts were approximately equal in size and the nucleated part was cut from the anterior end of the amœba.

At five minutes after division both fragments were attached and moving. Three minutes later the attachment in both was broken by the sliding of a small glass rod under them. The nucleated part began immediately to send out pseudopods aimlessly. This continued for two minutes; then the segment attached and remained so for three hours-when the experiment was brought to a close. This segment was attached continuously to the substratum; it appeared to be attached usually at three different points and the behavior appeared to be normal in every respect. The enucleated segment was momentarily attached at several different times, and each time the attachment was so weak that it could not resist even the slightest jar given the table on which the experiment was made. At no time was this segment continuously attached longer than two minutes and it was never attached at more than one point at a time. There was a slow streaming motion in the protoplasm but no locomotion at all except for a short time during its first attachment and then it was very slight. The results obtained in all of the observations on the attachment of segments of Amæba are briefly summarized in Table I. By referring to this table it will be seen that attachment of nucleated parts to the substratum is continuous: that attachment of enucleated parts is intermittent, slight and of short duration.

These facts seem to indicate that the nucleus directly influences the attachment of protoplasm to the substratum and thus influences behavior.

SUMMARY.

1. Amæba proteus moves regularly and smoothly by alternate formation of pseudopods on the two sides of the organism. Locomotion in segments of Amæba with a nucleus is of the same general character. Movement in segments without a nucleus, however, is irregular, jerky, very much slower than that in nucleated parts, and the pseudopods are not ordinarily formed regularly or alternately.

2. In a horizontal beam of light, normal specimens direct

their locomotion from the source of stimulation, i. e., they orient and are negative. Parts containing a nucleus respond in the same manner; those without a nucleus, however, do not orient.

3. The rate of locomotion varies greatly in different individuals. Large specimens move more rapidly than small specimens, the rate of locomotion bearing a fairly definite ratio to the size of the specimen. The rate of locomotion in nucleated segments bears essentially the same ratio to their size as it does in normal individuals. Segments without a nucleus show very little locomotion and this is always relatively very slow and irregular.

4. The size of the parts, the contractile vacuole, and the position which the parts occupied in the intact specimens before division seem to be in no way responsible for differences in the behavior of nucleated and enucleated parts of Amxba.

5. The only other known respect—aside from those mentioned —in which the two parts differ concerns the nucleus. Consequently the differences in the behavior of these parts are, in all probability, in some way related to the nucleus.

6. The regulatory influence of the nucleus on behavior in *Amæba* seems to be brought about by some sort of an influence upon the attachment of the organism to the substratum.

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